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Non-Linear Behavior of Protein and Tannin in Wine Produced by Cofermentation of an Interspecific Hybrid (Vitis spp.) and Vinifera Cultivar

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Abstract
Wines produced from red interspecific hybrid grape cultivars (Vitis spp.) typically have lower tannin than wines produced from vinifera cultivars, which can be attributed to the lower concentration of tannins and higher concentration of tannin binding proteins of interspecific cultivars. Tannin in wines produced from hybrid cultivars could potentially be increased by blending hybrids with vinifera. We hypothesized that blending of grapes prior to fermentation (cofermentation) should result in final wine tannin concentrations lower than wine tannin concentrations predicted from the individual components due to protein-tannin binding, but that this effect should be absent from monovarietal wines blended post-fermentation. To evaluate this hypothesis, over a two-year study, a high tannin V. vinifera cultivar (Cabernet Sauvignon) was blended with an interspecific hybrid (Marquette) at different ratios either before (cofermentation) or after fermentation. The tannin and protein concentrations of the wines were measured by methyl cellulose precipitation assay and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), respectively. Tannin and protein concentrations in blended wines were compared to values predicted from the linear combination of the two monovarietal wines. Co-fermented blends with a high proportion of Marquette had up to 25% lower tannin than predicted, but for most cofermentations and post-fermentation blends observed and predicted tannin concentrations did not differ. However, protein concentrations for many of the blends – especially from cofermentation - were lower than the predicted values, in some cases >50%. Loss of protein due to adsorption to tannin was well modeled by a Freundlich absorption isotherm.

Keywords
blended wine, interspecific hybrid, protein, tannin, wine composition

Comments
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Research Article

Non-Linear Behavior of Protein and Tannin in Wine Produced by Cofermentation of an Interspecific Hybrid (*Vitis* spp.) and *Vinifera* Cultivar

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Abstract: Wines produced from red interspecific hybrid grape cultivars (*Vitis* spp.) typically have lower tannin than wines produced from *vinifera* cultivars, which can be attributed to the lower concentration of tannins and higher concentration of tannin binding proteins of interspecific cultivars. Tannin in wines produced from hybrid cultivars could potentially be increased by blending hybrids with *vinifera*. We hypothesized that blending of grapes prior to fermentation (cofermentation) should result in final wine tannin concentrations lower than wine tannin concentrations predicted from the individual components due to protein-tannin binding, but that this effect should be absent from monovarietal wines blended post-fermentation. To evaluate this hypothesis, over a two-year study, a high tannin *V. vinifera* cultivar (Cabernet Sauvignon) was blended with an interspecific hybrid (Marquette) at different ratios either

before (cofermentation) or after fermentation. The tannin and protein concentrations of the wines were measured by methyl cellulose precipitation assay and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), respectively. Tannin and protein concentrations in blended wines were compared to values predicted from the linear combination of the two monovarietal wines. Co-fermented blends with a high proportion of Marquette had up to 25% lower tannin than predicted, but for most cofermentations and post-fermentation blends observed and predicted tannin concentrations did not differ. However, protein concentrations for many of the blends – especially from cofermentation - were lower than the predicted values, in some cases >50%. Loss of protein due to adsorption to tannin was well modeled by a Freundlich absorption isotherm.

Key words: blended wine, interspecific hybrid, protein, tannin, wine composition

Introduction

Interspecific hybrid grapes (*Vitis* spp.) are widely grown in the Northern USA and Canada due to their cold-hardiness and disease resistance. However, red wines produced from hybrid grape cultivars typically have low tannin concentration and poor mouthfeel as compared to wines produced from European wine grapes (*V. vinifera*) (Springer & Sacks 2014). Recent reports indicate that the lower tannin concentration of hybrid wines is likely due to the combined effect of lower skin tannins and higher concentrations of extractable proteins in hybrid grape cultivars (Springer and Sacks 2014, Van Sluyter et al. 2015). These tannin-binding proteins appear to be “pathogenesis-related proteins” of the same types responsible for white wine haze (Van Sluyter et al. 2015). Furthermore, the higher residual protein of hybrid wines results in lower retention of added exogenous tannin as compared to *V. vinifera* wines (Springer et al. 2016b). Strategies to mitigate the tannin-protein interactions in red wine, such as bentonite treatment before (Springer et al. 2016a) or during fermentation (Nicolle et al. 2019), have minor or insignificant effects on final wine tannin. The reasons for these minimal effects are unclear but may be because protein extraction and tannin binding continue throughout maceration (Bindon et al.

2016, Springer et al. 2016a). The poor retention of tannin can be compensated for through use of high rates of exogenous tannin addition, but this approach leads to undesirable sensory characteristics (brown color, off-aromas, bitterness) in the finished wine due to impurities in commercial products (Harbertson et al. 2012).

The blending of lower tannin hybrid grapes or wines with higher tannin *vinifera* cultivars (e.g. Cabernet Sauvignon) could potentially increase the tannin content of hybrid-based wines. Some investigations on post-fermentation blends of *vinifera* have reported final wine tannin concentrations intermediate to the monovarietal components, although this did not translate to differences in perceived astringency (Caceres-Mella et al. 2014, Dooley et al. 2012). A thorough investigation by Hopfer et al. on blends of monovarietal wines produced from Bordeaux cultivars reported that final concentrations of iron reactive phenols (IRP) were generally intermediate to blend components, but in some cases resulted in IRP concentrations higher than the component wines (Hopfer et al. 2012). However, it is not clear that these results can be extrapolated to blends of high protein, low tannin grapes (e.g. most hybrids) and high tannin, low protein grapes (many *vinifera*). As discussed above, addition of tannin to hybrid wines – analogous to post-fermentation blending – results in low tannin retention. However, pre-fermentation tannin additions to hybrid musts – analogous to co-fermenting two or more grape varieties – have also shown low recoveries of exogenous tannin, typically < 10% (Manns et al. 2013, Nicolle et al. 2019).

We hypothesized that final wine tannin concentrations should be lower in wines produced by cofermenting (CF) low tannin, high protein hybrids and high tannin, low protein vinifera grapes than by producing the wine by post-fermentation (PF) blending of the monovarietal wines. We expected that during CF, *vinifera*-derived tannins would bind to hybrid-derived proteins, decreasing their extractability. These proteins and other potential tannin-binding compounds (e.g. polysaccharides) could potentially be removed by post-fermentation racking, such that PF blending would yield higher final wine tannin. In this work, we investigated the effects of blending type (CF vs. PF) on both the protein

and tannin concentrations. The binding of protein between tannin and protein was also examined using the Freundlich model, as described in previous work (Springer et al. 2016b).

Materials and Methods

Chemical Reagents. Ammonium sulfate (granular, certified ACS) and SYPRO Ruby protein gel stain were obtained from Fisher Scientific (Waltham, MA). Epicatechin (≥90%, HPLC), methylcellulose (viscosity: 1500 cP), β-mercaptoethanol (99%) were obtained from Sigma-Aldrich (St. Louis, MO). Potassium metabisulfite was purchased from Presque Isle Wine Cellars (North East PA). Concentrated sodium dodecyl sulfate solution, concentrated 4× Laemmli sample buffer were obtained from Bio-Rad Laboratories Inc. (Hercules, CA). Enzyme kits for determining L-malic acid (K-LMAL) and glucose/fructose (K-FRUGL) measurements were purchased from Megazyme (Wicklow, Ireland).

Raw Fruit Material. Marquette (Mq) grapes were sourced from the Iowa State UniversityHorticulture Research Farm (Ames, IA, USA) and harvested manually (1 Sept 2016 and 9 Sept 2017) at physiological ripeness. Whole clusters were divided into 2.27 kg bags and stored at -20 °C for three months. Cabernet Sauvignon (CS) from a Sonoma, CA vineyard was purchased as frozen must (previously destemmed and crushed) from Brehm Vineyards (Underwood, WA, USA) for both 2016 and 2017 growing seasons.

Winemaking. Mq grapes and CS must were thawed at 10 °C for 3 days before the Mq grapes were crushed and destemmed using a Corvina crusher-destemmer (Enotecnica Pillan, Rampazzo, Italy). The resulting Mq must (skins, pulp and juice) and thawed CS must (skins, pulp and juice) were weighed into 7.6 L plastic pails (6.8 kg total/pail) in varying ratios (Table 1). Fermentations were performed in triplicate except in the case of year 2, Trials 6 and 7, where the fermentations were performed in duplicate due to insufficient fruit.

Prior to blending, initial juice chemistry (soluble solids, pH, TA) was measured for the Mq and CS juices (supplemental information). Soluble solids were measured using a PAL-1 pocket refractometer (Atago, Bellevue, WA), pH was measured using an Orion 2-Star benchtop pH meter (ThermoFisher Scientific, Waltham, MA) and titratable acidity was measured using a Titrino plus automatic titrator (Metrohm, Riverview, FL). For Year 1 trials, sucrose was added proportionately to trials 1 through 4 to increase the sugar concentration to be equal to the Cabernet Sauvignon must. No must adjustments were made for Year 2. All fermentations were conducted at room temperature (approximately 20 °C).

In Year 1, Lalvin ICV D254 yeast were rehydrated with GoFerm (Scott Labs, Petaluma, CA) as per the manufacturer's instructions. Musts were inoculated at a rate of 0.2 g/L. After 24 h musts, were inoculated with lactic acid bacteria (Lalvin VP41; Scott Labs). Fermentations were all performed at 20 °C. Manual punch downs were performed daily. Specific gravity and temperature were monitored using a DMA (Anton Paar, Austria). After specific gravity was unchanged over two consecutive days, the musts were pressed on a bench-top stainless-steel fruit press (Brewcraft, Vancouver, WA, USA) into 3.78 L glass bottles fitted with an airlock and stored at 17 °C to complete fermentations. Residual sugar and malic acid were checked daily by enzymatic assay (Megazyme, Ireland) until alcoholic and malolactic fermentation were complete (residual sugar < 1.0 g/L, malic acid < 0.3 g/L). Wines were then racked into a clean 3.78 L glass bottle with a screwcap and supplemented with potassium metabisulfite (50 mg/L as SO_2). At this stage, the three fermentation replicates were combined into two lots before analysis or post-ferment blending in order to minimize headspace in the 3.78 L storage bottles. After three months of dark storage at 10 °C, the wines were racked again. After 12 days, wines were bottled into 750 mL clear glass screw cap bottles using an Enolmatic Vacuum Filler (Tenco, Italy). Bottled wine was stored in the dark at 4 °C until analysis. Tannin analysis was performed approximately 1 year after bottling, and protein analysis was performed approximately 1.5 years after bottling. Timing

differences of the two analyses (tannin and protein) were due to lags in method development and validation. However, we observed that tannin and protein concentrations results changed negligibly in the periods between the two analyses.

Year 2 fermentations were conducted similarly to Year 1 fermentations, with the following changes: all fermentations (except 100Mq rep 1, 100Mq rep 2 and 50Mq50CS rep 3) were pressed after 9 days of skin contact when the fermentations were at apparent dryness (sp gr < 1). The other three fermentations were pressed after 14 days of skin contact, at which point they had reached apparent dryness. Immediately after pressing, the wines were evaluated for residual sugar and malic acid, and all were determined to be finished fermenting. The wines were racked into 3.78 L glass bottles with a screwcap after 24 h settling at 10 °C and supplemented with potassium metabisulfite (30 mg/L SO_2) to give a final dose of 30 mg/L sulfur dioxide. The lower dose of SO_2 in year 2 as compared to year 1 was the result of a clerical error but was not expected to affect the outcome of this study. After two months, the wines were bottled approximately 750 mL clear glass screw cap bottles. Bottled wine was stored dark at 4 °C until analysis. Tannin analysis was performed after approximately 3 months and protein analysis was performed after approximately 4.5 months.

Tannin Quantitation. Initial attempts to quantitate tannins using the Adams-Harbertson protein precipitation assay were unsuccessful because tannin concentrations in many wine samples were below the assay detection limit (Harbertson and Adams 2000). Wine tannins were instead measured using the methyl cellulose precipitation (MCP) assay (Mercurio and Smith 2008). The assay was modified slightly, in that the final volume of the control and sample tubes was 5 mL instead of 10 mL, i.e. all reagent and sample volumes were decreased by one half. A calibration curve was created using a series of epicatechin standard solutions in water ranging from 0 to 200 mg/L. Spectrophotometric analysis was performed on a Genesys 10S UV-vis spectrophotometer (ThermoFisher Scientific, Waltham, MA). Predicted tannin for wine blends was calculated from the proportional contribution of the 100% Mq and

100% CS tannin concentrations:

Equation 1 $Predicted\ Tannin = a * \%Mq + b * \%CS$

Where *a* is the concentration of tannin in 100% Mq wine and *b* is the concentration of tannin in 100% CS wine. Calculated values are available in Supplemental Tables 1 and 2.

Protein Quantification by SDS PAGE. Sample preparation and SDS-PAGE experiments were performed according to a previous report (Springer et al. 2016b). After dialysis, samples were concentrated using a Savant SpeedVac Plus (ThermoFisher Scientific, Waltham, MA) and stored at -80 °C until further experiments. For analysis, samples were thawed at room temperature and reconstituted in 500 µL ultrapure water. A 30 µL aliquot of sample was added to 60 µL loading buffer (BioRAD 4x Laemmli Sample buffer with 10% β-mercaptoethanol added), vortexed and heated to 95 °C for 5 minutes. After cooling to room temperature, the samples were centrifuged at 10000 g for 1 minute using an accuSpin Micro centrifuge (Fisher Scientific, Hampton, NH) before loading a 20 µL subsample onto a Mini-PROTEAN TGX Precast 12% glycine gel. Mark12 Unstained Standard (Life Technologies, Thermofisher Scientific, Waltham, MA) was included in each run. Gel electrophoresis was performed using a Bio-Rad Mini-PROTEAN Tetra cell at constant conditions (400 A, 70 mV) for approximately 115 minutes. Gels were stained overnight in SYPRO Ruby protein gel stain, and fixed in a 10% methanol, 7% acetic acid solution for 45 to 60 minutes before washing and storing in water.

For protein quantitation, gels were imaged with a Typhoon FLA 9500 scanner (GE Healthcare, Chicago, IL) and processed using ImageQuant software (GE Healthcare, Chicago, IL). Two bands from the standard ladder (Bovine Serum Albumin 66.3 kDa and Trypsin Inhibitor, 21.5 kDa) were chosen for all other bands to be normalized against, based on the manufacturer's stated concentrations for these proteins. Five distinct and consistent bands were integrated for each sample and summed to give an approximate total protein concentration. Predicted protein for wine blends was calculated from the proportional contribution of the 100% Mq and 100% CS wines, analogous to tannin quantitation.

Calculated values are available in Supplemental Tables 3 and 4.

Modelling Protein Adsorption to Tannin. The adsorption of proteins to condensed tannins was modeled using the linear form of the Freundlich adsorption isotherm:

$$\text{Equation 2} \qquad \log (q_s) = \log K_F + b_F \log [C_s]$$

where q_s is the mass ratio (mg/mg) of protein adsorbed to the amount of condensed tannin in a volume of wine, C_s is the equilibrium concentration of protein remaining in the wine (mg/L), K_F (L/mg) is an empirical constant that represents the adsorption capacity of the adsorbing agent (tannin), and b_F is dimensionless and represents the adsorption intensity. The protein adsorbed component of q_s was calculated from the following equation:

$$\text{Equation 3} \quad qq_{ss} = \frac{}{PPPPPPPPPPPPPP_{pppppppppppppppppp}[TTTTPPPPPPPP_{pppppppppppppppppp} - [PPPPPPPPPPPPPP]\ _{ooooooppppoopppp}]}$$

A plot of log (q_s) vs log [C_s] was generated used Graphpad Prism, and K_F and b_F were determined from the intercept and slope, respectively.

Statistical Analysis. Tannin and protein concentrations for the CF and PF wines were compared using separate t-tests for each set of proportional wines using GraphPad Prism version 7.00 for Windows (La Jolla, CA). For these t-tests, the Bonferonni-Dunn correction was applied with an initial p-value of 0.05 to account for non-equivalent standard deviations. The tannin and protein concentrations for the CF and PF wines were then compared to the predicted values using one-way t-tests by setting the predicted concentrations as the known value. The one-way t-tests were performed using the GraphPad QuickCalcs web site (https://www.graphpad.com/quickcalcs/).

Results and Discussion

Two cultivars (Cabernet Sauvignon and Marquette) were used in different blending proportions for cofermentation (CF) and post-fermentation (PF) experiments, summarized in Figure 1. Cabernet

Sauvignon (CS) was chosen as a representative high tannin *vinifera* cultivar (Harbertson et al. 2008). Marquette (Mq) was chosen as the low tannin interspecific hybrid variety because of its interest to upper Midwest and Northeastern states and Canada due to its extreme cold hardiness and positive aroma and flavor characteristics (Manns et al. 2013). Basic juice and wine chemistry for the monovarietal, unblended wines are shown in Table 2. The Mq juice in year 1 had moderate sugar concentrations and acid levels. The final ethanol concentration in the year 1 Mq wine of 13.9% v/v is consistent with many commercial red wines. In contrast, the CS juice had a very high sugar concentration, which resulted in high final ethanol (17.4% v/v) and was the likely cause of slower fermentation kinetics (data not shown). Beaver et al. recently reported that increasing ethanol concentration from 0 to 15% resulted in a 10-fold increase in the proanthocyanidin (tannin) extraction equilibrium constants for proanthocyanidin-CWM model systems (Beaver et al., 2019). The effect on ethanol on the equilibria of proanthocyandins between CWM and wine for the more limited range of ethanol concentrations in our current experiment is unknown, but is expected to be smaller. For year 2, both the Mq and the CS juice were similar in terms of sugar concentrations, which led to ethanol concentrations of 14.5% v/v and 13.4% v/v respectively. The year 2 fermentations did not show any differences in fermentation kinetics (data not shown) and were all pressed off after the same number of days with skin contact.

Condensed tannins were measured in monovarietal wines and their blends. As expected, tannin concentrations in 100% CS wines were ~5-fold higher than 100% Mq wines (~2000 vs. ~400 mg/L as epicatechin equivalents). The 100% CS values (1921 mg/L and 2270 mg/L for year 1 and 2 respectively) were comparable to previous reports (Mercurio and Smith 2008). There are no previous reports of measuring condensed tannin in hybrids using the MCP assay, but a previous study reported a tannin concentration in Mq wine of 48 mg/L based on phloroglucinolysis HPLC (Manns et al. 2013), which is well below typical concentrations reported in vinifera wines by phloroglucinolysis - HPLC, 119 to 376 mg/L (Kennedy et al. 2006). Predicted tannin and protein concentrations were also determined for the

blends based on the assumption that final concentrations would be the weighted average of the components. For both CF and PF wines, tannin concentration increased with increasing CS content in the wine for both growing years (Figure 2A and 2B). For year 1, CF tannin concentrations in all treatments did not differ significantly from predicted values. For year 2, only CF wines produced with the highest proportion of Mq had significantly lower tannin than predicted, specifically the 90% Mq/10% CS (-24.9% as compared to predicted). PF tannin concentrations did not differ significantly from values predicted from the monovarietal components.

The lower-than-predicted tannin for the 90% Mq/10% CS cofermentation blending (but not postfermentation blending) may be due to adsorption of the CS tannin onto Mq proteins. Recent work has demonstrated that tannin extractability and retention vary considerably among grape sources (Bindon et al. 2014, Fragoso et al. 2011), even under identical fermentation conditions, and can be particularly low in *Vitis spp.* hybrids (Springer et al. 2016a). The low extractability/retention in hybrids was credited to their higher concentrations of tannin-binding soluble proteins, which are detectable in the juice prior to fermentation and may be further extracted during fermentation (Springer et al. 2016a). As discussed in more detail below, the high concentration of protein in the unblended Mq wine (Table 2) supports this hypothesis. In the case of PF wines, the agreement between predicted and observed tannin may be because tannin-protein binding is cooperative and does not follow fixed ratios (Waterhouse et al. 2016). Therefore, a large portion of the Mq protein could have been lost in the PF treatment following its vinification, pressing, and racking, and prior to its contact with tannin in the CS wine. However, under cofermentation conditions, this Mq protein would be able to bind CS-derived tannins. Our PF blending results contrast with reports that blending leads to concentrations of iron-reactive phenols (IRP) which were occasionally greater than the IRP predicted from individual blend components (Hopfer et al. 2012). This earlier work used only *vinifera* cultivars (Cabernet Sauvignon, Merlot and Cabernet franc) all which have relatively high tannin concentrations in comparison to Marquette for their subsequent wines

(Harbertson et al. 2008, Mercurio and Smith, 2008, Caceres-Mella et al. 2014). Another report evaluated PF blends of wines produced from Cabernet Sauvignon, Carménère, Merlot and Cabernet Franc (Caceres-Mella et al. 2014). The authors of these papers did not perform calculations to determine if flavanol or tannin concentrations in blended wines were similar to values predicted from the unblended components; however, inspection of the raw data from these papers suggests this was the case.

Protein concentrations were determined in wines by SDS-PAGE and staining, and a representative gel is shown in Figure 3. Protein bands were observed at 14, 23 to 29, and 65 kDa (Figure 3), and are likely the same pathogenesis-related proteins identified in previous studies on red hybrid wines and white wine haze: chitinase, thaumatin like protein (TLP) and invertase (Van Sluyter et al. 2015, Springer et al. 2016b). The 100% Mq wine had ~3-fold higher protein than the 100% CS wine in both years, and thus was the major contributor of protein to the blended wines. For most blends and years, protein concentrations were lower in both CF and PF blends as compared to the amount predicted based on weighted averages of the component wines (Figure 2). For example, the year 1 50Mq50CS wines (both PF and CF) had 50 to 60% the protein of predicted values (4.3 mg/L and 4.7 mg/L vs. 8.2 mg/L respectively, $p=0.05$). Thus, blending of Mq with CS results in a loss of protein as compared to the monovarietal components, presumably due to greater binding of hybrid-derived proteins by *vinifera*derived tannins.

Vintage variation was not explicitly investigated during this work but some differences in the significance of treatments were observed across years. For example, tannin in the year 2 CF wine 90Mq10CS was significantly lower than the predicted concentration, whereas in year 1 it was not. Similarly, protein in the year 2 PF wine 50Mq50CS was significantly different from the predicted concentration, whereas in year 1 no significant differences were observed. The reasons for these inconsistencies are unclear. However, it is known that other must components, e.g. cell wall material (CWM) can bind tannin (Beaver, et al. 2019). These components were not measured in the current

study, but if they varied due to vintage factors (temperature, precipitation, etc.) they could affect final concentrations of tannins and proteins.

For the selected cultivars, we observed clear negative deviations from linear behavior for protein in blended wines, but only minor or non-significant effects on tannin in blended wines. We also observed that the tannin concentration is approximately 40-fold greater than the protein concentration in the 100Mq wines and 400-fold greater in the 100CS wines (Table 1). Based on the fact that protein appeared to be limiting, a Freundlich adsorption isotherm was used to model the adsorption of tannin onto proteins (Pedneault et al. 2013, Springer et al. 2016b) As seen in Figure 4, the linearized Freundlich equation is good model of final concentrations of protein as a function of tannin concentration ($r^2 > 0.98$ in both years). These observations suggest that for the combination of grapes selected (Mq and CS), tannin is excess of protein, and that final tannin and protein concentrations are well described by a cooperative binding model. Potentially, selection of *vinifera* and hybrid pairs with lower tannin and higher protein (e.g. Lemberger and Maréchal Foch in a previous report) (Springer et al. 2016a) would have yielded the opposite result, i.e. greater negative deviation from non-linear behavior for tannin in blends, although this was not evaluated in our current work.

Although they were not part of this experimental design, several viticultural or winemaking decisions could additionally affect the extent of protein and/or tannin extractability, both in covinifications or ordinary vinifications. For example, sunlight exposure can lead to higher overall phenolic concentration in grapes (Scharfetter et al., 2019), and full deficit irrigation as compared to standard industry irrigation practices has been demonstrated to significant increase tannins in Cabernet Sauvignon wines (Cassassa et al., 2015). Some protein can be removed before fermentation by bentonite fining of juice (Springer et al., 2016a) or tannin extraction, and specifically seed tannin extraction, can be amplified through lengthened maceration times (Cerpa-Calderón, 2008). Factors that

increase the amount or rate of tannin extraction or decrease protein content should result in higher final tannin and lower wine protein.

Conclusion

We observed that cofermentation of a high-tannin cultivar (CS) with a low-tannin cultivar (Mq) results in lower final tannin concentration than values predicted from a proportional contribution of the monovarietal components. This effect is proposed to be due to decreased tannin extractability during maceration as a result of protein-tannin interactions. This hypothesis supported by the presence of lower than predicted protein concentrations in blended wines. Significant differences were not observed between predicted and experimental tannin concentrations for post-fermentation blends, suggesting that post-fermentation blending may be preferable for ensuring a higher tannin concentration when producing blended wines from high and low tannin cultivars.

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Table 1 Proportional composition of the experimental wines.

Trial #	Year 1		Year 2	
	% Mq	% CS	% Mq	% CS
1	100	0	100	0
2	90	10	90	10
3	75	25	75	25
4	50	50	60	40
5	0	100	50	50
6			40	60
7			0	100

Table 2 Chemical analysis of the monovarietal Mq and CS juices and wines.

		Soluble Solids (Brix)	pH	Titratable Acidity (g/L)	Ethanol (% v/v)	Residual Sugar (g/L)	Volatile Acidity* (g/L)	Days on Skins	Tannin (mg/L epicatechin equiv.)	Protein (mg/L)
Year 1										
Marquette	juice	24.3	3.28	6.9	-	-	-	-	-	-
	wine		3.40	5.9	13.9	0.4	0.32	7	474 ± 128	12 ± 1.4
Cabernet Sauvignon	juice	29.6	3.91	6.1						
	wine		3.87	6.3	17.4	7.3	0.82	13	1922 ± 178	4 ± 0.23
Year 2										
Marquette	juice	25.1	3.24	7.8	-	-	-	-	-	-
	wine		3.33	7.7	14.5	0.0	0.46	9	358 ± 42	19.8 ± 3.7
Cabernet Sauvignon	juice	24.5	3.63	4.3	-	-	-	-	-	-
	wine		3.48	6.8	13.4	0.0	0.47	9	2270 ± 91	5.1 ± 0.9

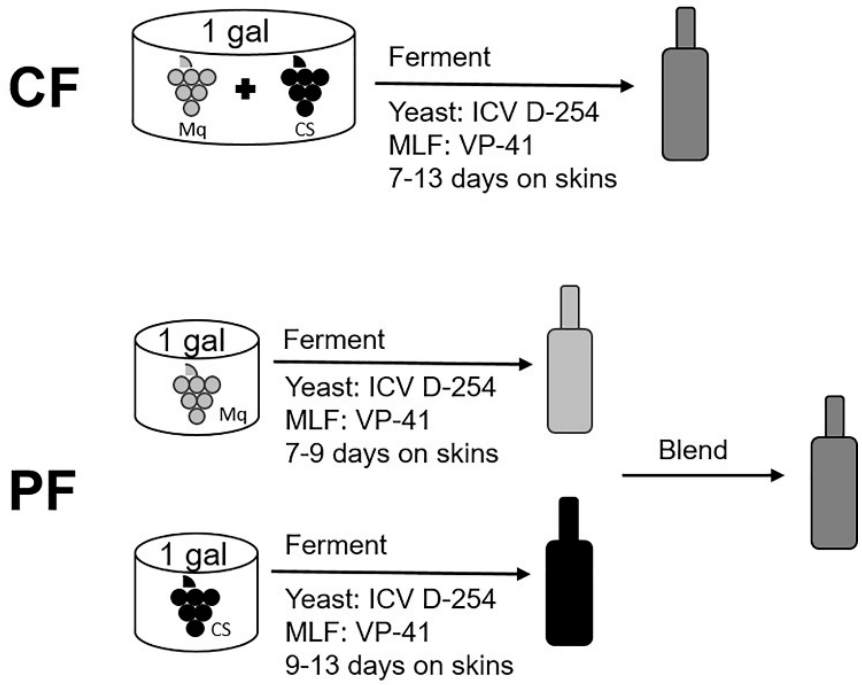

Figure 1 Experimental summary for the cofermentation (CF) and postfermentation (PF) blending experiments. Mq = Marquette, CS = Cabernet Sauvignon.

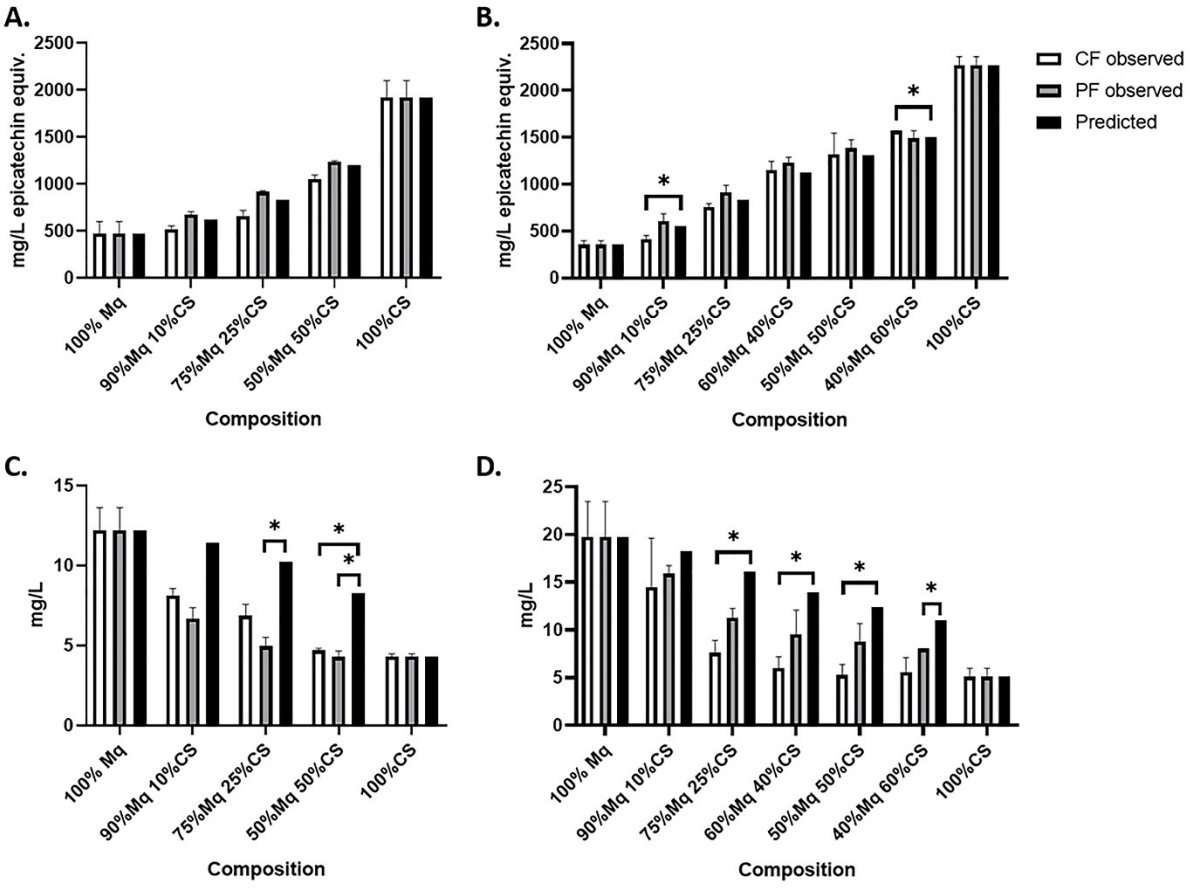

Figure 2 Tannin and protein concentrations in finished wines produced by blending Marquette (Mq) and Cabernet Sauvignon (CS) in different proportions. Blending was either performed following crushing and cofermented (CF) or performed on wines postfermentation (PF). (**A**) Year 1 tannin concentrations. (**B**) Year 2 tannin concentrations. (**C**) Year 1 protein concentrations. (**D**) Year 2 protein concentrations. Statistically significant differences are identified with a (*) as determined by t-tests ($p = 0.05$).

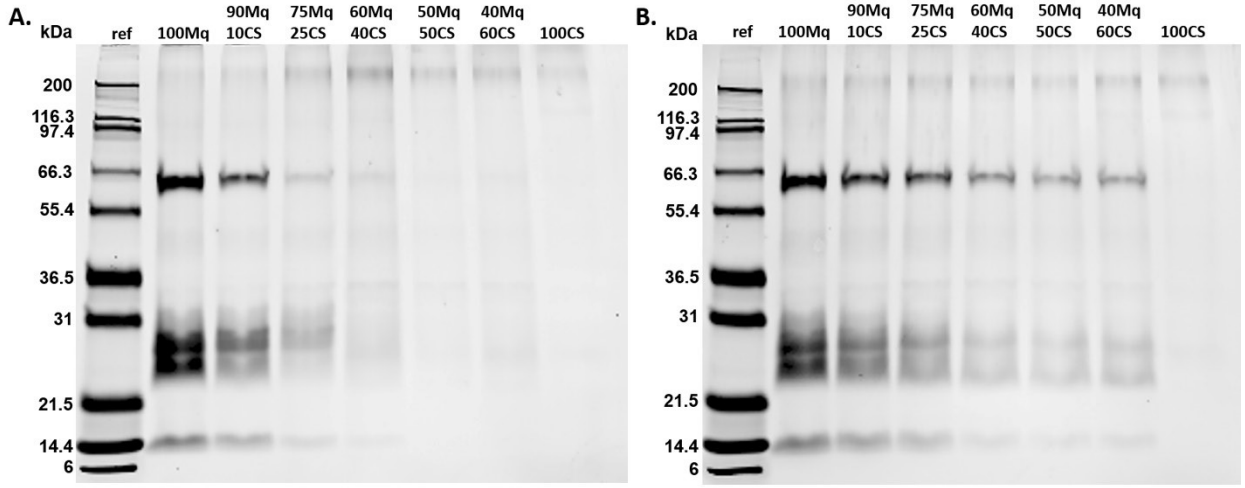

Figure 3 Exemplary SDS-PAGE gels (both Year 2) of proteins isolated from wines made by blending Marquette (Mq) and Cabernet Sauvignon (CS) in various proportions (denoted at the top of each lane). (**A**) Cofermentation, (**B**) Postfermentation blending. For quantification the bands at 66.3 kDa and 21.5 kDa of the reference standard ladder in the first lane were normalized based on the manufacturer's reported concentrations.

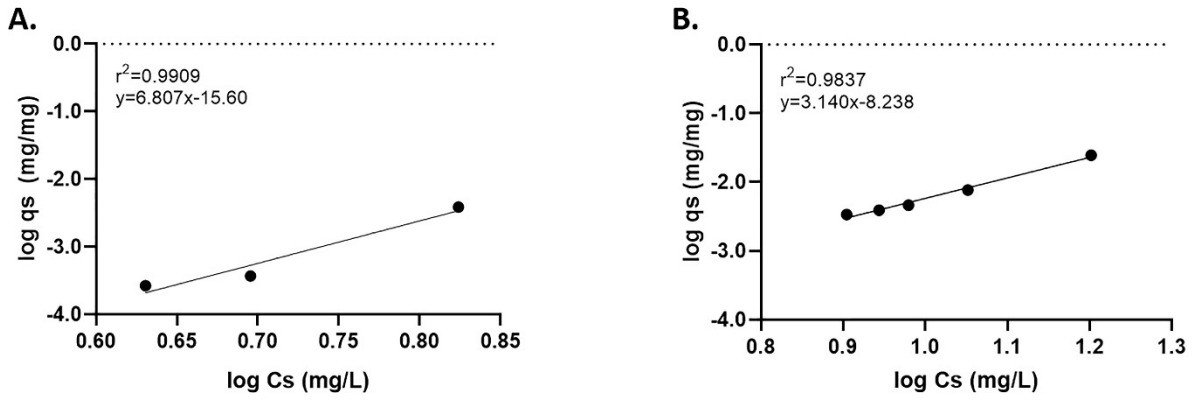

Figure 4 Linearized Freundlich adsorption isotherm at ambient conditions (*ca.* 20 °C) illustrating the adsorption of tannins onto proteins. These curves were generated with tannin and protein concentrations observed in wines made from post-fermentation blending of Marquette and Cabernet Sauvignon in different proportions. (**A**) Year 1 (**B**) Year 2. The log transformation of the mass ratio of protein adsorbed to the amount of tannin in wine (q_s) is plotted on the y-axis vs. the log transformation of equilibrium wine protein (C_s) on the x-axis.

Supplemental Table 1 Tannin concentrations for year 1. Coferment (CF) measurements are shown for both fermentation replicates. Measurements expressed as mg/L epicatechin equivalents. PF = postfermentation blending, stdev = standard deviation.

Sample Ratio		CF-Y1 F1		CF-Y1 F2		PF-Y1		Predicted
Marquette (% Mq)	**Cabernet Sauvignon (% CS)**	**mg/L**	**stdev**	**mg/L**	**stdev**	**mg/L**	**stdev**	**mg/L**
100	*0*	383	33	564	4	474	128	474
90	*10*	546	156	495	9	501	85	619
75	*25*	701	20	610	7	788	27	836
50	*50*	1027	49	1084	18	1142	20	1198
0	*100*	1796	27	2048	94	1922	178	1922

Supplemental Table 2 Tannin concentrations for year 2. Coferment (CF) measurements are shown for all fermentation replicates. Measurements expressed as mg/L epicatechin equivalents. PF = postfermentation blending, stdev = standard deviation. *For CF-Y2 F3, only 2 replicates were performed due to insufficient grape material.

Sample Ratio		CF-Y2 F1		CF-Y2 F2		CF-Y2 F3		PF-Y2		Predicted
Marquette (% Mq)	**Cabernet Sauvignon (% CS)**	**mg/L**	**stdev**	**mg/L**	**stdev**	**mg/L**	**stdev**	**mg/L**	**stdev**	**mg/L**
100	*0*	336	2	406	34	331	38	358	42	358
90	*10*	458	60	404	46	374	89	607	22	549
75	*25*	777	22	780	65	719	55	874	58	836
60	*40*	1148	34	1065	26	1248	17	1104	29	1123
50	*50*	1279	22	1116	22	1563	2	1294	10	1314
40	*60*	1568	34	1572	5	*	*	1529	41	1505

0	*100*	2334	81	2206	34	*	*	2270	91	2270

Supplemental Table 3 Protein concentrations for year 1. Coferment (CF) measurements are shown for both fermentation replicates. PF = postfermentation blending, stdev = standard deviation.

Sample Ratio		**CF-Y1 F1**		**CF-Y1 F2**		**PF-Y1 R1**		**PF-Y1 R2**		**Predicted**
Marquette (% Mq)	**Cabernet Sauvignon (% CS)**	**mg/L**	**stdev**	**mg/L**	**stdev**	**mg/L**	**stdev**	**mg/L**	**stdev**	**mg/L**
100	*0*	13.7	1.4	10.3	0.8	12.1	1.3	12.6	1.0	12.2
90	*10*	7.8	1.6	8.4	0.5	7.2	0.8	6.2	0.7	11.4
75	*25*	7.4	0.7	6.3	0.8	5.3	0.9	4.6	0.7	10.2
50	*50*	4.8	0.7	4.6	0.6	4.5	0.8	4.0	0.6	8.2
0	*100*	4.2	1.3	4.3	0.6	4.5	0.9	4.1	0.6	4.3

Supplemental Table 4 Protein concentrations for year 2. Coferment (CF) measurements are shown for all fermentation replicates. PF = postfermentation blending, stdev = standard deviation. *For CF-Y2 F3, only 2 replicates were performed due to insufficient grape material.

Sample Ratio		**CF-Y2 F1**		**CF-Y2 F2**		**CF-Y2 F3**		**PF-Y2 R1**		**PF-Y2 R2**		**Predicted**
Marquette (% Mq)	**Cabernet Sauvignon (% CS)**	**mg/L**	**stdev**	**mg/L**	**stdev**	**mg/L**	**stdev**	**mg/L**	**stdev**	**mg/L**	**stdev**	**mg/L**
100	*0*	20.4	1.4	14.1	1.3	22.4	1.9	23.4	9.3	18.5	2.0	19.8
90	*10*	13.2	0.7	10.1	1.1	20.1	2.3	15.3	2.1	16.5	2.6	18.3
75	*25*	7.9	0.9	6.2	0.8	8.7	1.7	11.9	1.5	10.6	1.0	16.1
60	*40*	6.5	1.1	4.6	0.5	6.9	0.9	11.3	0.6	7.7	0.2	13.9
50	*50*	5.0	0.4	4.4	0.6	6.5	0.6	10.1	2.0	7.4	0.9	12.4

40	*60*	6.6	1.1	4.5	0.5		8.0	1.1	8.0	0.8	11.0
0	*100*	5.0	0.4	3.9	0.5		5.9	1.0	5.6	0.2	5.1